

Claims:

1. Calibration standard for determining base proportions of degenerated bases in DNA, wherein a degenerated base represents at least two different bases in at least two DNA molecules at the same position, wherein said standard is produced by a process comprising the steps of:
 - providing at least two DNA molecules or DNA pools being not identical and containing at least two bases of the degenerated base at different positions within at least one DNA molecule; and
 - mixing the DNA molecules or DNA pools in unequal ratios, thereby obtaining the calibration standard.
2. Calibration standard according to claim 1, wherein the at least two DNA molecules or pools thereof are provided by chemically synthesizing different oligonucleotides in separated synthesis.
3. Calibration standard according to claim 2, wherein the oligonucleotides are amplified and cloned.
4. Calibration standard according to claim 1, wherein the at least two DNA molecules are provided by supplying inhomogeneous DNA and cloning it, determining the base composition differences of at least two DNA molecules or pools thereof and selecting the at least two DNA molecules or pools thereof.
5. Calibration standard according to claim 4, wherein the inhomogeneous DNA is supplied by providing genomic DNA containing single nucleotide polymorphisms (SNPs).
6. Calibration standard according to claim 4, wherein the inhomogeneous DNA is supplied by chemically synthesizing an oligonucleotide containing degenerated bases.
7. Calibration standard according to claim 4, wherein the inhomogeneous DNA is provided by chemically treating DNA containing unmethylated cytosine bases in

such a way that the unmethylated cytosine bases are converted to uracil, whereby said conversion is incompletely performed and statistically evenly distributed, and optionally, the obtained modified DNA is amplified.

- 5 8. Calibration standard according to claim 7, wherein the chemical treatment is conducted with a bisulfite (= disulfite, hydrogen sulfite).
9. Calibration standard according to claims 4 to 8, wherein the inhomogeneous DNA is amplified.
- 10 10. Calibration standard according to claim 1, wherein at least three DNA pools are provided.
11. Calibration standard according to claim 1, wherein DNA pools are provided
15 containing each base of the degenerated base at different positions within each DNA molecule.
12. Calibration standard according to claim 1, wherein the degenerated base represents two bases, preferably cytosine and thymine.
- 20 13. Calibration standard according to claim 1, wherein the DNA molecules contain at least 40 degenerated bases, preferably at least 145 degenerated bases.
14. Calibration standard according to claim 1, wherein DNA molecules are provided
25 showing an identity of less than 60% at the positions having implemented bases of the degenerated base, preferably less than 23%.
15. Calibration standard according to claim 1, wherein DNA molecules are provided showing the lowest possible identity at the positions having implemented bases of
30 the degenerated base.
16. Calibration standard according to claim 1, wherein the DNA molecules are mixed in a ratio based on a numerical series of b^n , b being the number of bases of the degenerated base, and n being the set of nonnegative integers from 0 to the
35 difference of the number of provided DNA molecules and 1.

17. Kit comprising a calibration standard according to claims 1 to 16 and optionally, instructions for use of the kit.

5 18. Use of a calibration standard according to claims 1 to 16 for determining base proportions of degenerated bases in DNA.

19. Method for determining base proportions of degenerated bases in DNA, a degenerated base representing at least two different bases in at least two DNA molecules at the same position, comprising the steps of:

- 15 - providing trails each containing the DNA, a DNA polymerase, a sequencing primer with a label corresponding to any base moiety, 2'-monodeoxy-NTPs, and a 2',3'-dideoxyanalog, whereby the 2'-monodeoxy-NTPs are contained in excess compared to the 2',3'-dideoxyanalog;
- DNA-dependent extension of the sequencing primer by a DNA polymerase, whereby fragments of different length with the dideoxyanalogs at the 3' terminus are obtained;
- unifying the trails;
- 20 - separating the fragments; and
- detecting the labels, thereby determining the base proportions of degenerated bases,

wherein the calibration standard of claims 1 to 16 is used.

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20. Method for calibration of measurement systems which determine base proportions of degenerated bases in DNA, wherein a degenerated base represents at least two different bases in at least two DNA molecules at the same position, characterized by the use of a calibration standard according to claims 1-16 comprising the steps of

- 30 - performing said measurement system with one or several of said calibration standards at least once,
- determining a calibration curve or function
- performing said measurement system with the sample to be analyzed
- comparing the result with those of step 1 and step 2 and
- 35 - assessing the measurement system performed.

21. Method according to claim 20 for calibration of measurement systems which determine the proportions of cytosine and thymine at positions which show a degenerated base following conversion of unmethylated cytosines, characterized by the use of a calibration standard according to claims 7-16 .

22. Method for production of a calibration standard for determining base proportions of degenerated bases in DNA, a degenerated base representing at least two different bases in at least two DNA molecules at the same position, comprising the steps of:

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- providing at least two DNA molecules being not identical and containing at least two bases of the degenerated base at different positions within at least one DNA molecule; and
- mixing the DNA molecules in unequal ratios, thereby obtaining the calibration standard.

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